Remarks

Claims 1-8, 10-13, 16-21, and 24-36 were pending in the subject application. By this Amendment, claims 1, 3-5, 10, 21, 24-30, 32, and 36 have been amended, claims 2, 6, and 11 have been cancelled, and new claims 37-41 have been added. Support for the new claims and amendments can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 1, 3-5, 7, 8, 10, 12, 13, 16-21, and 24-41 are currently before the Examiner for consideration. Favorable consideration of the pending claims is respectfully requested.

By this Amendment, claims 37-41 have been added. Support for claims 37-41 can be found, for example, at page 8, lines 13-15, and page 28, lines 17-20 of the specification.

Claims 1, 5, 10, 21, 25, 28, 29, and 31 are rejected under 35 USC §102(e) as anticipated by Yu *et al* (U.S. Published Application No. 2003/0186916). Yu *et al.* is cited as teaching a vector for transfecting a eukaryotic cell, comprising a nucleic acid, a nucleic acid binding polymer, and a lipid-based vesicle. Applicant respectfully submits that the Yu *et al.* publication does not teach the claimed subject matter. However, by this Amendment, Applicant has amended independent claims 1, 5, 10, and 21 to specify that the particle is a nanoparticle. Dependent claims 3, 4, 24-30, 32, and 36 have been amended similarly for antecedent basis. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §102(e) is respectfully requested.

Claims 1-8, 10-13, 16-21, 24-28, and 30-36 have been rejected under 35 USC §103(a) as obvious over Hart (2000) and further in view of Ni *et al.* (U.S. Patent Application No. 2002/0151009). Applicant respectfully traverses these grounds for rejection because the cited references, alone or in combination, do not disclose or suggest the claimed invention.

The Hart publication describes nanoparticles composed of cDNA and chitosan (page 203, section 3.4) and lipopolyplex vectors composed of a combination of a lipid and a cationic polymer (page 203, section 4). However, sections 3.4 and 4 of Hart point one skilled in the art toward the utilization of proteins and peptides to increase the transfection efficiency of these respective structures (*e.g.*, transferrin ligands; peptides derived from a histone, a nucleoline, or a protamine;

small viral peptides such as influenza virus haemagglutinin HA-2; apoE-3 fragment), not toward particles comprising a combination of chitosan or a chitosan derivative, a lipid, and a polynucleotide, as recited in the currently pending claims. In fact, the Hart publication indicates that in nanospheres incorporating chitosan, reporter gene expression in mice was higher and more sustained than that achieved by naked DNA and a lipid (LipofectAMINE) (page 203, section 3.4). This does not suggest that there would be any advantage to incorporating a lipid into particles composed of DNA and chitosan.

The Examiner references various paragraphs from throughout the Ni *et al.* publication in an attempt to show that Ni *et al.* teach a composition comprising all three of a polynucleotide, a lipid, and chitosan (or a chitosan derivative). However, Applicant respectfully asserts that Ni *et al.* does not disclose or suggest an embodiment wherein all three of a polynucleotide, a lipid, and chitosan (or a chitosan derivative) are disclosed within a single embodiment. At paragraphs 0410-0411 of the Ni *et al.* publication, the Examiner asserts that Ni *et al.* teach a compound comprising a nucleic acid and a lipid ("encapsulation in liposomes"). This section of Ni *et al.* does not teach or suggest a compound comprising chitosan and, therefore, does not teach or suggest all the elements of Applicant's claimed invention. In paragraph 1032 of the Ni *et al.* publication, the Examiner asserts that Ni *et al.* teaches a formulation comprising nucleic acid and chitosan. This section of the Ni *et al.* publication does not teach or suggest a composition comprising a lipid and, therefore, again does not teach or suggest all three elements of Applicant's claimed invention. The Examiner then references paragraph 1034 of the Ni *et al.* publication as teaching a composition comprising "release rate modification agents" which the Examiner asserts includes "pore-forming agents such as fatty acids (lipids)." However, Applicant respectfully asserts that this section of the Ni *et al.* publication does not teach or suggest the combination of all three of the elements: a polynucleotide, a lipid, and chitosan (or a chitosan derivative) in a particulate form. Thus, the Ni *et al.* publication does not teach or suggest the elements of independent claims 1, 10, 17, and 21, and claims dependent therefrom.

In regard to claims 2, 6, and 11 (now cancelled, and incorporated into independent claims 1, 5, and 10, respectively), the Examiner asserts that it is well-established in the art that the delivery of

nucleic acids in particles comprising chitosan or liposomes is in the nanoscale. However, a particle of chitosan, *etc.*, does not necessarily have to be in the nanoscale. Moreover, Applicant respectfully submits that there is no explicit teaching or suggestion in the Ni *et al.* publication of a particle comprising a polynucleotide, a lipid, and chitosan (or a chitosan derivative) that is in the nanoscale.

Neither of the cited references suggests that a combination of chitosan and a lipid into a single particle would be desirable or would improve transfection efficiency beyond that provided by chitosan and lipids individually. On the contrary, the inventor surprisingly found that such a combination does, in fact, achieve enhanced transfection *in vivo*.

Example 3 of the subject specification describes an experiment conducted to determine the transfection efficiency of chitosan-lipid nanoparticles in target lung epithelial cells. Groups of BALB/c mice were administered intranasally with 25μg of total pEGFP DNA (plasmid encoding green fluorescent protein (GFP)) complexed with chitosan, Lipofectin (a cationic liposome preparation), or in chitosan-lipid nanoparticles. Figure 3C shows a graphical representation of the results in which bar "1" indicates transfection with chitosan alone, bar "2" indicates transfection with Lipofectin alone, bar "3" indicates transfection with chitosan-lipid nanoparticles, and bar "4" is DNA alone. As shown by this comparative data, chitosan-lipid nanoparticles induced a 30% transfection rate in lung cells compared to the ~20% transfection rate induced by chitosan and Lipofectin alone, demonstrating that chitosan-lipid nanoparticles represent a more efficient delivery system.

As indicated in the Manual of Patent Examining Procedure (MPEP 716,02(a)), "a greater than expected result is an evidentiary factor pertinent to the legal conclusion of obviousness ... of the claims at issue." *In re Corkill*, 711 F.2d 1496, 226 USPQ 1005 (Fed. Cir. 1985). Evidence of a greater than expected result may also be shown by demonstrating an effect that is greater than the sum of each of the effects taken separately (*i.e.*, "synergism"). *Merck & Co. Inc. v. Biocraft Laboratories Inc.*, 874 F.2d 804, 10 USPQ2d 1843 (Fed. Cir.), *cert. denied*, 493 U.S. 975 (1989). Evidence of unobvious or unexpected advantageous properties, such as superiority in a property the claimed compound shares with the prior art, can rebut *prima facie* obviousness. "Evidence that a compound is unexpectedly superior in one of a spectrum of common properties . . . can be enough to

rebut a *prima facie* case of obviousness." No set number of examples of superiority is required. *In re Chupp*, 816 F.2d 643, 646, 2 USPQ2d 1437, 1439 (Fed. Cir. 1987).

Not only did the chitosan-lipid nanoparticles exhibit increased transfection efficiency, but they also show a decreased induction of IL-6, a pro-inflammatory cytokine, when compared to chitosan alone. Example 4 of the subject specification compares the effect of chitosan-lipid nanoparticles on IL-6 level, relative to chitosan and Lipofectin individually. Mice were intranasally given vector plasmid pVAX complexed with chitosan, with Lipofectin, or in chitosan-lipid nanoparticles, and IL-6 production was examined after 4 hours. Figure 4 shows a graphical representation of the results, which demonstrate that IL-6 levels were significantly reduced when using chitosan-lipid nanoparticles compared to chitosan alone and, therefore, represent a safer gene delivery system.

Not only do the cited references fail to suggest combining chitosan and lipids in a gene delivery system, they give no hint of the unexpected benefits conferred by their combination as demonstrated in the subject specification. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §103(a) is respectfully requested.

It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicant's agreement with or acquiescence in the Examiner's position.

In view of the foregoing remarks and amendments to the claims, Applicant believes that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

Applicant invites the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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